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Commentary

POFUT1 and *PLAGL2* gene pair linked by a bidirectional promoter: the two in one of tumour progression in colorectal cancer?

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Colorectal cancer (CRC) is a major health burden that can be well managed if the tumour is detected at an early stage. To identify novel biomarkers for early diagnosis, an increasing number of studies have focused on gene expression dysregulations in the colon and rectal cancers, as in CRC cell lines. Several studies revealed that the 20q11.21 chromosomal region is amplified in at least 65% of CRC cases [1]. Such a genomic instability leading to amplification is also found in numerous other cancer types, including breast cancer, lung cancer, melanoma, and numerous others [2]. The shortest amplified portion associated with acute myeloid leukemia only contains four genes including pleomorphic adenoma gene-like 2 (*PLAGL2*) and protein *O*-fucosyltransferase 1 (*POFUT1*) [3]. Their expressions have been particularly investigated in the context of CRC by the team of Xiaorong Li, published in the July issue of *EBioMedicine* [4].

PLAGL2 is a zinc-finger transcription factor located in the nucleus, whose expression shown to be significantly higher in CRC tissues than in adjacent non-tumour tissues [5]. In intestinal organoid cultures, *PLAGL2* has shown to drive intestinal epithelial stem cell expression signature, which then activates *WNT* gene expression and enhances TCF/LEF reporter. Overall, *PLAGL2* overexpression in CRC promotes *in vitro* and *in vivo* cell proliferation by the Wnt/β-catenin pathway [6].

POFUT1 is an ER-resident glycosyltransferase that allows fucose addition on S or T included in the C2X4(S/T)C3 consensus motif, where C2 and C3 are the second and third cysteines out of six conserved in EGF-like domain. The major known target of POFUT1 is NOTCH receptor, which contains 14 to 20 *O*-fucosylation consensus sites according to the four human paralogs; most of the 20 sites on NOTCH1 are *O*-fucosylated [7]. *POFUT1* is overexpressed in colorectal tumours compared to non-tumour adjacent tissues and positively regulates colorectal tumour progression through activation of Notch signaling pathway [8,9].

Both *PLAGL2* and *POFUT1* overexpression in CRC can be associated to copy number amplification. In the article by Li and colleagues [4], several experimental data are given to support the hypothesis that their correlated expression is driven by the activity of a bidirectional promoter. First, the authors showed that the adjacent head-to-head genes are separated by a short promoter sequence that is evolutionarily conserved in mammals. In 2015, the downregulation of *Pofut1* in *Plagl2* −/− mice had already led Chen and colleagues [10] to consider that the two genes likely share a common promoter region. Interestingly, the conservation of the bidirectional promoter would mean that under physiological conditions, coordinated regulation of both genes' expression is essential, at least in mammals. This opens the possibility to reproduce the amplification of the two genes in CRC animal models *i.e.* mouse and to test the efficiency of modification of the transcription factor binding sites to attenuate the overexpression of the two genes and thus carcinogenicity, without altering their essential physiological functions [4]. Next, the demonstration of the bidirectional activity of the promoter was done *in vitro* using reporter gene constructs and CRC cell lines. Upon silencing *PLAGL2*, *POFUT1* or both in HCT116 and SW480 cells, the authors were able to show that downregulation of the gene pair synergistically reduced colorectal tumourigenesis. Further studies will be required to understand the mechanism leading to this co-expression and biological implications within the tumour environment. Indeed, the tumour tissue is complex because of its cellular heterogeneity compared to a cell line. Tumour is certainly not subjected to the same potential epigenetic regulations, nor to the expression of the same transcription factors compared to cancer cell lines. Another interesting finding is that the correlated overexpression of *PLAGL2* and *POFUT1* initiates deregulation of the two signaling pathways, Wnt/β-catenin and Notch, and subsequently a deregulation of the cell cycle and tumour progression [4]. Further studies are needed to specify the respective contributions of Wnt/β-catenin and Notch signaling in promoting CRC and in maintaining the stemness of CRC stem cells. This study also raises the question of whether, for other cancers, the two genes cooperate in the same way as in the CRC. While it is fairly easy to imagine that overexpression of a transcription factor, like *PLAGL2,* may modulate several gene expressions, it is a bit more complicated to figure out how overexpression of *POFUT1* encoding an *O*-fucosyltransferase, modifies the expression of genes that affect the cell cycle and the cellular proliferation and invasion. It is widely accepted that *O*-fucosylation of Notch, by POFUT1, affects Notch signaling. However, it remains to be determined what is the *O*-fucosylation state of NOTCH receptors in the colorectal tumour. Moreover, NOTCH receptors are not the only targets of POFUT1

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and it is not excluded that other *O*-fucosylated actors participate in the consequences of *POFUT1*overexpression in CRC.

Finally, this work contributes to the understanding of the mechanisms involved in tumour progression and to the identification of potential biomarkers. In connection with human health, it is rather conceivable to use this type of data for the purpose of improving diagnostics, but it seems more premature to project on a therapeutic approach focusing on the bidirectional promoter region.

Declaration of Competing Interest

The authors declare no conflict of interest.

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